THE ASSOCIATION OF *RHIZOCTONIA BATATICOLA* WITH RETTING FLAX IN SOUTH AUSTRALIA.

By D. B. Adam and Joan Stokes, Waite Agricultural Research Institute.
(*Communicated by Dr. H. L. Jensen.*)

[Read 21st October, 1942.]

"Dew retting" is essentially a biological process wherein pectic substances which help to bind the fibre bundles together are decomposed by the action of fungi.

Jensen (1941) has recently dealt with the micro-organisms found on Victorian flax during retting, and he concludes that the fungi *Cladosporium herbarum*, *Dematium pullulans* and *Alternaria* spp. are the most active organisms under natural conditions of "dew retting". Jensen also carried out experiments which showed that the relative predominance of any species among the various "retting fungi" was influenced greatly by temperatures during the course of retting, and that this effect was reflected both in the time taken to complete retting and in the quality of the product. Control of temperature seems, then, to offer an opportunity to improve the efficiency of "dew retting".

Experiments with this object in mind were started, and it was during their progress that the fungus discussed in this paper was first observed. The flax straw used in most of the work was grown at the Waite Institute, though the same fungus has been observed on flax straw from other South Australian localities. After a wetting secured by immersion in water for periods of from 2–8 hours, bundles of flax about 12 lb. in weight were kept in cabinets maintained at a constant temperature. In flax kept at 90°F. or 80°F. we observed that after a period of from 65–137 hours some of the straws, particularly at their lower ends, acquired a pale greyish appearance and were densely covered with the minute sclerotia of a fungus. The fibre bundles in affected parts were, or within a day or two became, almost completely disintegrated. Commercially speaking, such straws were badly "over-retted" and so weak as to be useless for spinning purposes. The proportion of straw affected varied from sample to sample; it was greatest in flax held at 90°F.; at 80°F. it was only slightly less evident. The same fungus has also been observed in flax held at 60°F. and at 100°F. and in straw spread in the field during the winter months, as is normally done for "dew retting" locally. However, at a temperature of 60°F. the fungus constituted a small proportion, and at 100°F., or under ordinary field conditions, a very small proportion, of the fungal flora present on the retting flax. It does not appear likely that the activity of this sclerotial fungus will contribute much to loss in quality of the fibre product under ordinary "dew retting" conditions.

Isolation, Artificial Culture and Some Physiological Characteristics of the Fungus.

The fungus is easily isolated from infected straw on a variety of media. We used Starkey's modification of Waksman's acid agar (Starkey, 1929), in which the essential nutrients are glucose, 10 grams, and peptone, 5 grams per litre. Growth of the fungus exhibits the same general characteristics on a wide variety of media. The mycelium is white and grows rapidly at favourable temperatures. At 30°C. a 9 cm. Petri plate is covered in 2–3 days. Within a short time the fungus produces numerous roughly round sclerotia. On Dox's agar their mean diameter varied from 63–165μ with a mean of 97μ. On potato dextrose agar they varied from 50μ–181μ, mean 113μ. Other aspects of those sclerotia whose production and appearance on a variety of media is very characteristic, are dealt with later.

We have tried to induce sporulation by growing the fungus on a variety of artificial media, including media with and without yeast extract, on sterile flax stems and other plant materials and on soil extract agar, but neither in the case of any artificial medium nor on naturally-infected flax carrying a mixed flora, have we observed any spore forms that could be associated with the fungus.

Some Aspects of the Physiology of the Fungus.

(a) Growth in Relation to Temperature.

The abundance of the sclerotial fungus in flax maintained in a cabinet at 90°F. indicated a definite temperature preference and suggested a need for detailed information on the temperature relations of the fungus. The method of investigation consisted of growing the fungus in pure culture on potato dextrose agar (P.D.A.) plates, exposing them to various temperatures and measuring the increase in colony diameter from time to time. It was necessary to standardize certain details in this procedure. We found, for instance, that the rate of growth on P.D.A. plates at low temperatures tended to increase slightly after some time, whereas at optimal and higher temperatures the reverse was true. A concomitant of this was the apparent effect of the size of a colony when experimental conditions were first imposed, on the rate of growth over the following 24 hours. These effects, however, are of minor importance in the wider picture of temperature effects and, while taken into account in securing results, need not be discussed further. Table 1 summarizes the effect of temperature on the growth rate on P.D.A. plates of the sclerotial fungus from flax. Each figure is the mean derived from 12–14 observations. It was not practicable to secure all data in a single experiment, but care was taken to standardize technical details (amount of media, size of dishes, etc.) and the time and manner in which measurements were made on each of the three occasions over which it was necessary to spread the experiment.

TABLE 1.

Mean Diameter Increase over 24-Hour Period of a Sclerotial Fungus from Retting Flax. Grown on Potato Dextrose Agar Plates at the Temperatures Shown.

Temp. °C.	14·3	15·3	17·5	20·0	25·0	30·0	32·5	34·1	35·8	37·0
Diam. increase (mm.)	5·6	10·0	15·2	19·6	33·3	45·3	49·0	32·0	23·6	10·8

Above 37° C. mycelial growth becomes strongly aerial and in consequence the measurement of colony diameter difficult.

At 40° C., which is about the maximum, growth ceases within 24 hours.

The optimum temperature for growth falls rather sharply within the range 30°–32·5° C. This is a high optimum for what is apparently, as evidence given later suggests, a soil-inhabiting fungus in a temperate climate.

(b) Capacity to utilize Carbonaceous Food Materials.

The rapidity and completeness with which this fungus achieves the "retting" of flax raises the question of its ability to utilize the various carbonaceous food materials present in flax straw, especially cellulose, of which the fibres are composed. The question was investigated with pure cultures of the fungus grown on Müntus basal NH_4NO_3 agar (Ashby, 1927), to which various carbonaceous materials were added in uniform quantities of 15 grams per litre. The carbonaceous supplements used were: dextrose, xylose, soluble starch (B.D.H.), pectin powder 100 grade rapid setting (B.D.H.) and cellulose. As the source of cellulose the requisite weight of filter-papers (Whatman's No. 2) was macerated in dilute sulphuric acid, washed and suspended in the basal medium.

The results of this work are expressed in terms of the number of sclerotia per microscopic field (diam. 0·31 mm.) after three weeks' incubation at 27°C. This period allowed ample time for sclerotial production to reach a settled state.

The figures in Table 2, which summarize the results of this work, are each means calculated from observations of 30 microscopic fields. The selection of fields for observation was so arranged that systematic variation in sclerotial production on a plate, from the centre of the colony to its outer edge and between replicate plates, could be estimated and separated from the estimate of standard error.

TABLE 2.

Mean Number of Sclerotia per Standard Unit Area with Different Carbonaceous Nutrients.

Pectin.	Dextrose.	Xylose.	Cellulose.	Starch.	No Carbonaceous Supplement.
29·3	28·3	28·0	26·9	23·3	0

Difference for significance, 3·24
(nil series excluded).

On the plates receiving no carbonaceous supplement, while a sparse growth of mycelium reaching the edge of the plate occurred, it was not vigorous enough to form any definite sclerotia.

The number of sclerotia per unit area, however, does not on its own provide a complete picture of the value of these different materials for growth. It takes no account of the size of sclerotia that develop and hence no complete idea of the mass of such material produced. The sizes of sclerotia on the plates we are concerned with are given in Table 3. They are mean figures each based on 120 measurements.

TABLE 3.

Mean Diameters (μ) of Sclerotia produced on Agar Plates with Different Carbonaceous Nutrients.

Pectin.	Dextrose.	Xylose.	Cellulose.	Starch.
83·2	115·7	101·7	78·6	87·3

Difference for significance, 1·32.

The results given above reveal that the fungus can utilize each of the above carbonaceous materials though the extent of growth as measured by sclerotial production varies.

If we combine the data of Tables 2 and 3 with a view to considering the suitability of the food material used in terms of the mass of sclerotial tissue produced, it is evident that dextrose is the best, with xylose slightly less so. The other three materials are definitely less suitable with but little differences between them.

One way in which they do differ should, however, be mentioned. On the cellulose plates the sclerotia were fairly evenly distributed from the centre to the edge of the plates, but in all the other plates they were significantly more numerous at the edges rather than in the centre.

(*c*) *Relation to Acidity.*

The sclerotial fungus was grown on a series of plates of a modified synthetic cotton root agar medium (Luthra and Vasudeva, 1938), the pH of which was varied by additions of malic acid or sodium carbonate. In this way nine series of plates whose initial pH ranged from 3·4 to 10·6 were obtained. Growth was measured in terms of increase in colony diameter over 24 hours. The plates were incubated at 27°C.

Good growth at practically an equivalent rate occurred over the pH range 5·0–8·0. Below pH 5·0 it fell slowly at first and then more rapidly. At pH 3·4 it was about one-quarter the rate at the optimum. On the alkaline side the fall in growth rate over a 24-hour period was not so rapid. At pH 10·6, the most alkaline media tested, it was still rather more than half the optimum. It was evident that the fungus could grow over a very wide range of pH.

Identity of the Fungus.

We recall the fact that neither on naturally-infected straw nor on a variety of substrates designed to promote sporulation have we succeeded in inducing the formation of spore bodies which would facilitate identification. Affinities for this fungus must then be sought for among the group of non-sporing *Fungi imperfecti.*

We consider that the fungus should be referred to *Rhizoctonia bataticola* (Taub.) Butler.

It is true that certain strains of *R. bataticola* are referable to a sphaeropsidaceous species, *Macrophomina phaseoli* (Maubl.) Ashby (Taubenhaus, 1913), but it is equally evident from the extensive literature on the subject that a connection between many isolates of *R. bataticola* and *M. phaseoli* has not been established. The fungus from retting flax apparently falls within this category. We summarize the evidence in favour of the proposal to refer this fungus to *R. bataticola* in the following paragraphs:

(1) *Morphological Characters.*—The colour and manner of growth of the mycelium, the mode of formation, character, size and abundance of sclerotia produced by the fungus in culture agree with descriptions (Taubenhaus, 1913; Luthra and Vasudeva, 1938) and figures (Taubenhaus, 1913; Ashby, 1927; Luthra and Vasudeva, 1938) of *R. bataticola.*

For comparative purposes we tried to secure an authentic culture from Ceylon,* but this was not available. Access to material at Baarn, Holland, is, of course, impracticable at present.

(2) *Physiological Characters.*—The temperature relations and the tolerance to acidity and alkalinity on solid media of the fungus from flax are similar to those described for *R. bataticola* (West and Stuckey, 1931; Luthra and Vasudeva, 1938).

The fungus from flax can utilize cellulose, a quality which it shares in common with sterile strains of *R. bataticola* in Ceylon (Gadd—see footnote). On the other hand, strains having the capacity to reproduce the pycnidial stage, *M. phaseoli*, have been reported as apparently unable to decompose cellulose (West and Stuckey, 1931).

(3) *Pathological Characters.*—Taubenhaus (1913), when first describing *Sclerotium bataticola* Taub., now referred to *Rhizoctonia bataticola* (Taub.) Butler, showed that the fungus, under suitable circumstances, could induce symptoms of "charcoal disease" in sweet potatoes after an incubation period of three to eight weeks. We have conducted similar experiments and have been able to induce "charcoal rot" in injured sweet potatoes incubated at 25°C. for a period of four weeks.

R. bataticola has also been reported to cause a disease of jute seedlings. Our own attempts at producing a disease in these plants were unsuccessful. The failure may have been due either to the fungus used being non-pathogenic or to some lack in the environmental conditions necessary to infection (West and Stuckey, 1931).

Discussion.

The markedly greater prevalence of this sclerotial fungus on the basal parts of mature flax plants suggests that the fungus originates from the soil. The unspecialized character of saprophytism of this fungus has been shown locally by its occurrence on the mature stems of weeds growing in the flax crop. Further evidence of its relation to soil and plants other than flax was provided years ago by S. D. Garrett,† who, in the course of studying the fungus flora of healthy mature wheat plants, isolated a similar fungus which he also referred to *R. bataticola.*

R. bataticola appears, then, to be present naturally in Waite Institute soil, and since it has been observed on flax from other parts of South Australia, is probably quite a common inhabitant of soils in this State. This occurrence of *R. bataticola* is notable since published records of its geographical distribution show that while widely distributed in tropical countries, e.g., Formosa, Ceylon, East Africa and the south-eastern United States, it has not attracted attention in more temperate regions.

Summary.

The occurrence of a sclerotial fungus on retting flax is described. It was most evident on flax maintained for retting at temperatures of 90°F. It also occurs, but not freely, on flax being "dew retted" in the field. When this sclerotial fungus develops vigorously, it quickly induces "over-retting" and destroys the quality of the fibre.

Morphological, physiological and pathological characters of the fungus have been studied, and on this basis the fungus has been referred to *Rhizoctonia bataticola.* This fungus, which is widely known in tropical countries, is considered to be a natural element in some, and possibly in a wide range of, South Australian soils.

---

* We are indebted to Drs. Eden and Gadd for help in this matter and to the latter for animadversions on "What is *R. bataticola*?".

† Unpublished records, Waite Agricultural Research Institute.

Bibliography.

ASHBY, S. F., 1927.—*Macrophomina phaseoli* (Maubl.) comb. nov. the Pycnidial Stage of *Rhizoctonia bataticola* (Taub.) Butl. *Trans. Brit. Mycol. Soc.*, 12, 141-147.

JENSEN, H. L., 1941.—Microbiological Investigations on the Dew-Retting of Flax. PROC. LINN. SOC. N.S.W., 66, 276-286.

LEVINE, M., and SCHOENLEIN, M. S., 1930.—A Compilation of Culture Media for the Cultivation of Microorganisms. Monographs on Systematic Bacteriology. The Williams and Wilkins Company, Baltimore.

LUTHRA, J. C., and VASUDEVA, R. S., 1938.—Studies on the Root-Rot Disease of Cotton in the Punjab. v. Confirmation of the Identity of *Rhizoctonia bataticola*. *Ind. J. Agric. Sci.*, 8, 727-734.

STARKEY, R. L., 1929.—Some Influences of the Development of Higher Plants upon the Microorganisms in the Soil: I. Historical and Introductory. *Soil Sci.*, 27, 319-334.

TAUBENHAUS, J. J., 1913.—The Black Rots of Sweet Potato. *Phytopathology*, 3, 159-166.

WEST, J., and STUCKEY, W. R., 1931.—*Macrophomina phaseoli* (Maubl.) Ashby in Trinidad. Part I. Parasitism. Part II. Physiology. *Mem. Imp. Coll. Trop. Agric. Trinidad* (*Mycol. Ser.*), 4, 20 pp.